

## Acaricidal effects of essential oils from *Rosmarinus officinalis L*, and *Mentha spicata L* against *Varroa destructor* (Mesostigmata: Varroidae) agent of the varroatoose bee.

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### ABSTRACT

The parasitic *Varroa destructor* of the honey bee *Apis mellifera intermissa* is considered currently the greatest threat in this insect. The first attacks are hardly noticeable. It will sometimes take three years before we notice the first symptoms. From this time, the infestation can progress very quickly and lead to a sharp increase in mortality rates. In the present study, the toxicity of essential oils from *Rosmarinus officinalis L* and *Mentha spicata L* extracted by hydrodistillation was tested on *Varroa destructor*. In order to study the effect of these plants, three lots were formed at an apiary in Mitidja. The first batch is treated with the essential oil of rosemary, the second batch with the mint. The third batch has not undergone any treatment. The results show mortality and a relatively high efficiency with an average rate of 65.40 % and an efficiency of 95.89% for rosemary. For mint oil, we found an average rate of 56.44 % mortality and an efficiency of 97.38%. However, for the batch three untreated; the mortality rate of dead mites in a natural way throughout the treatment period is estimated at 56.65%. Therefore, essential oils have significantly reduced the initial infestation rate which was higher compared to that recorded at the end of treatments for all colonies.

**KEYWORDS:** *Varroa destructor*, *Apis mellifera*, essential oil, hydrodistillation, toxicity, *Rosmarinus officinalis L*, *Mentha spicata L*, infestation rates.

### INTRODUCTION

Various pollinators species including 20,000 species of bees in the world contribute to the survival and evolution of more than 80% of plants species [7]. The environment and agriculture are dependent on this insect. The latter has indeed an undeniable economic interest. We obviously think of bee products but above all has its fundamental role in pollination and, therefore in the preservation of the majority of plant species. Also, the bee is considered the strong link of animal life on earth. Its disappearance would lead undoubtedly to serious problems to nature. For decades, the honeybee population in the world is facing a real slaughter. This scourge is caused by the presence of an ectoparasite. *Varroa destructor*, Oudemans (1904) is a mite visible to the naked eye and is passed from bee Asian (*Apis cerana*) to honeybees (*Apis mellifera*) in the early 1980. Since then, the pest has won all of Western Europe and North Africa and causes considerable damage in apiaries which infests [21]. It attacks the workers and adult bees, but also the larvae. The female prolific varroa lays the eggs in the brood cells, and young parasitize the larvae to grow at their expense. Following its expansion to the bees

around the world, it was recently identified as a major factor that led to significant losses in the colonies [4-6-8]. It is in this perspective that the research in this area should focus on the means of fighting to implement. For ten years, beekeepers have access to a new line of research in the application of natural products such as essential oils from medicinal and aromatic plants that remain an inexhaustible source of biologically active substances and have biological properties very interesting that find application in various fields: medicine, pharmacy, agriculture, beekeeping ... .. [5-11]. These "biological" treatments have a more irregular efficiency. However residues present less harmful to health and can broaden the range of treatments and reduce the appearance of varroa resistance. In Algeria, all attempts of chemical and natural treatments have been used since its appearance [2-9]. Any time according to our knowledge, few works that are interested in the natural fight - based essential oils against Varroa in local conditions. However Koumad (2011 ) evaluated some ways to fight chemical and biotechnical against this parasite.

During our study, we initially were interested in determining the infestation rate in all the colonies and to highlight the acaricide activity of essential oils of rosemary and mint in order to develop a struggle against varroa [1].

## MATERIAL AND METHODS

### Material:

In this study, *Apis mellifera intermissa* breed of bee colonies are treated against the ectoparasite *Varroa destructor*. These bees are housed in modern hives Langstroth model. The treatments used are based on essential oils of rosemary and spearmint. The experimental apiary is located at Mitidja coastal Algerian center.

### Methods:

#### *Varroa diagnostic methods in bee colonies:*

In our experiment, a diagnosis by biological method or bottom covers was performed. On all hives, we ask for diapers coated with fat which is spread and for 15 days. They are then removed and examined carefully using a hand lens to count the dead mites naturally. Parallel to infection of dead mites, estimate the number of bees in each hive is made. Once the estimated number of varroa operations (c) and the number of bees (p) are made, the rate of initial infestation of each colony is determined by the following equation:

$$D^{\circ}IF = \frac{C}{P}$$

In order to get the final degree of infestation (D ° IF), the same processes for the laboratory diagnosis after treatment was resumed.

#### *Treatments for varroa with essential oils:*

##### *Actual treatment:*

After harvesting honey, acaricide products based on essential oils extracted by hydrodistillation are administered in all items by the same technique, treatments began on May 31. The experimental device is divided into 3 lots:

- Batch 1: five colonies treated with essential oil of rosemary then the Apiguard. This last is applied as control to verify the effectiveness of this treatment.
- Batch2: five hives treated with the essential oil of mint then the Apiguard.
- Batch 3: the control group. It consists of five untreated hives. It tracks the evolution of the parasitosis by placing daily diapers to collect dead varroa.

Varroa mortality surveys are daily from  $d_0$  à  $d_{16}$ , and the mortality rate is calculated as follows:

$$\text{mortality rate (\%)} = \frac{\text{number of dead varroa from } d_0 \text{ à } d + 31}{\text{number of varroa present in the colony}}$$

We also evaluated the level of oil efficiency on Varroa using the following formula:

$$\% \text{ efficiency} = \frac{\text{number of dead varroa from } d_0 \text{ à } d + 16}{\text{number of dead varroa from } d_0 \text{ à } d + 31}$$

##### *Control processing:*

This type of control treatment is intended to monitor the effectiveness of essential oils used immediately after the said treatment. This is done with the Apiguard. The latter is a frost thymol-based similar natural thymol.

All our results presented in all of our studies have been a statistical analysis by the variance analysis method. This analysis of variance was performed using the STATISTICA software to determine the degree of significance between the plant and the treatment period.

**Results:**

**Diagnostic results:**

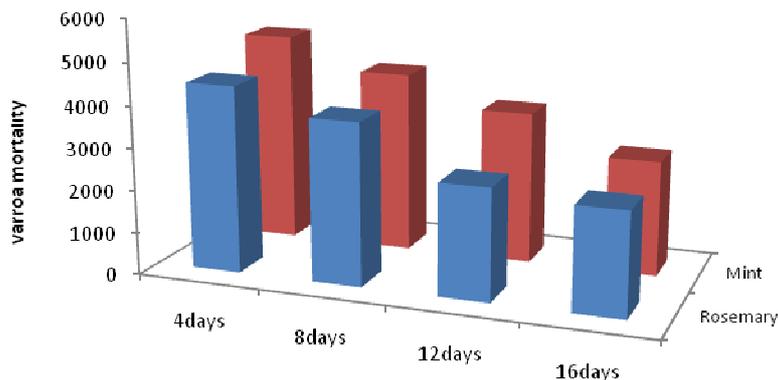
The results presented in Table 1 show that all colonies are infected with varroa. The degree of infestation on all the colonies is heterogeneous; it varies between 3.96% and 42.76%.

**Table 1:** Initial colony infestation rate

Batch	Hives	Number of varroa C	Number of bees P	D°II(%) C/P	Middle D°II(%)
Batch 1 (Rosemary)	H1	7356	43795	16.80	11.99
	H2	3665	27948	13.11	
	H3	2668	21064	12.67	
	H4	1105	27888	3.96	
	H5	5606	41733	13.43	
Batch 2 (Mint)	H6	3641	39312	9.26	14.67
	H7	6905	32843	21.02	
	H8	2422	55025	4.40	
	H9	6822	49545	13.77	
	H10	8692	34912	24.90	
Batch 3 (Witness)	H11	7941	18571	42.76	33.24
	H12	8753	25173	34.77	
	H13	8628	37187	23.20	
	H14	5852	18598	31.47	
	H15	7249	21306	34.02	

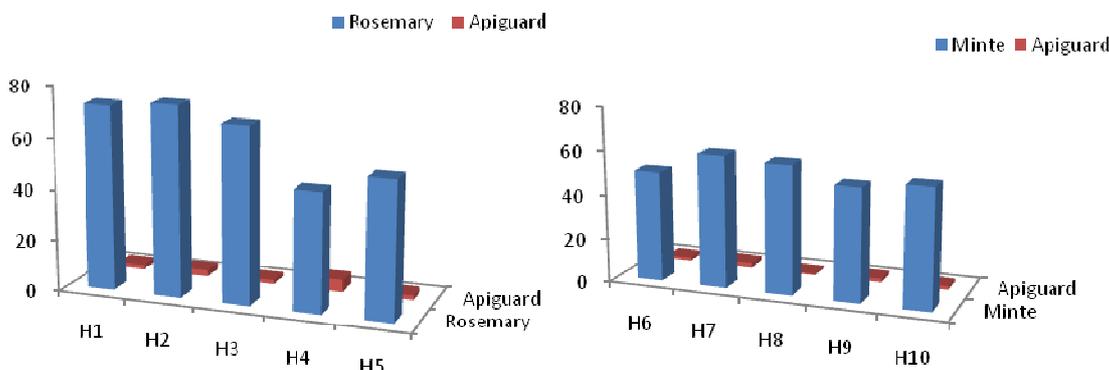
**Effect of essential oils on varroa:**

Following an initial application of essential oils of rosemary and mint, relatively high mortality was observed. Then she began to gradually decrease for the two lots and that over time (Fig. 1)



**Fig. 1:** Development of varroa mortality in batches over time.

The combined action of the essential oil and Apiguard spawned a mortality rate of 65.40% and 56.44% respectively for Lot 1 and lot2. The Apiguard alone is originally low mortality (Fig. 2a and Fig. 2b). Efficiency of the two treatments is almost similar in all hives with an average of 95.89% for rosemary and 97.38% for Mint (tab. 2)



**Fig. 2a:** Mortality rate of varroa induced with essential oils from Rosemary and Apiguard

**Fig. 2b:** Mortality rate of varroa induced with essential oils from Mint and Apiguard

**Table 2:** Mortality rate and efficiency of the essential oil of rosemary and mint

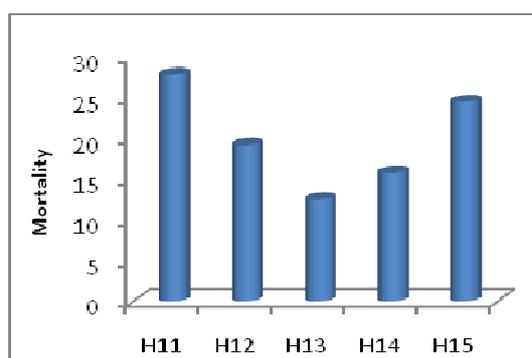
		Lot 1 : essential oil of Rosemary					Lot 2 : essential oil of mint				
		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Number of dead varroa from d <sub>0</sub> to d+16 with oils	1 <sup>ère</sup> app	1758	1023	555	185	908	651	1171	513	1246	1445
	2 <sup>ème</sup> app	1411	887	458	136	952	468	1143	512	904	1276
	3 <sup>ème</sup> app	1079	555	318	98	593	380	1113	320	698	1089
	4 <sup>ème</sup> app	1053	252	497	93	545	327	729	73	667	924
Total number of dead varroa from d <sub>0</sub> to d+16		5031	2717	1828	512	2998	1826	4156	1418	3515	4734
Number of dead varroa with «APIGUARD»	1 <sup>ère</sup> app	109	87	41	52	90	50	116	10	99	109
	2 <sup>ème</sup> app	30	5	0	1	8	0	24	2	26	30
Total number of dead varroa with «APIGUARD» from d <sub>16</sub> to d+31		139	92	41	53	98	50	140	12	125	139
Total number of dead varroa from d <sub>0</sub> to d+31		5440	2809	1869	565	3096	1876	4296	1430	3640	4873
Number of varroa present in the colony		7356	3665	2668	1105	5606	3641	6905	2422	6822	8693
Mortality rates (%) from both treatments		73.95	76.64	70.05	51.13	55.23	52.52	62.22	59.04	53.36	56.06
Middle mortality rate(%)		65.40					56.44				
Efficiency of the oil (%)		97.44	96.72	97.81	90.62	96.83	97.33	96.74	99.16	96.56	97.14
Middle efficiency de (%)		95.89					97.38				

*Varroa mortality for the untreated Lot:*

Natural mortality of varroa in batch three is 56.65% on average, the hive n° 13 présente a lower rate with an average of 40.21% due to its low rate of infection is 23. 20 %. The hive11 has more deaths throughout the trial period; it is the most infested lot with an initial infestation rate of 42. 76%. (tab. 3 and fig 3).

**Table 3 :** Natural rate mortality

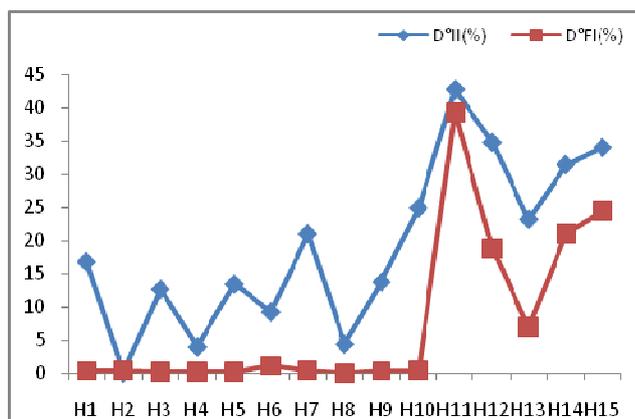
N° of hives	Number of varroa présent in the colony	Number of dead varroa from d <sub>0</sub> to d+16)	number of dead varroa from d <sub>16</sub> to d+31)	Total number of dead varroa	Mortality rates (%)	Middle mortality rate(%)
H11	7941	822	4518	5340	67.24	56.65
H12	8753	1458	3115	4573	52.24	
H13	8628	1439	2031	3470	40.21	
H14	5852	975	2568	3543	60.54	
H15	7249	593	3976	4569	63.02	

**Fig. 1:** Development of hives mortality in the witness.*Estimation of the final infestation rate:*

From Table 4 and after treatment with essential oils the average rate of final infestation is 0.31% and 0.47% respectively for batch 1 and batch 2. So, treatment has reduced the rate initial infestation (Fig. 4). The bee populations have also decreased.

**Table 4:** Final colony infestation rate

Hives	Number off varroa C	Number of bees P	D°IF(%) C/P	Middle D°IF (%)
H1	139	38960	0.36	0.31
H2	92	23658	0.39	
H3	41	17866	0.23	
H4	53	21394	0.25	
H5	98	39588	0.25	
H6	50	4559	1.10	0.47
H7	140	30029	0.47	
H8	12	26235	0.05	
H9	125	43936	0.29	
H10	139	32615	0.43	
H11	4518	11506	39.26	22.1
H12	3115	16588	18.77	
H13	2031	28999	7	
H14	2568	12258	20.94	
H15	3976	16259	24.45	



**Fig. 4:** Comparison between initial and final rate infestation of the hives

On analysis of variance results is carried out shows that during the various treatments, lot rosemary oil has a significant mortality of varroa in time and only one application is sufficient to exert a varroicide effect. ( $P < 0.01$ ). As for treatment with peppermint oil, changing kills varroa is not significant since the probability is greater than 0.01 indicating that mortality is the same way during the time of treatment and equal the control treatment.

Effect	Significance test from the essential oil of mint				
	SC	Degrees of freedom	MC	F	P
Ord. origine	10387729	1	10387729	93.59998	0.000000
Application	2482679	4	620670	5.59263	0.003448
Error	2219601	20	1109805		

Sc :squared som. Mc : means square. F : critical value. P : probability

Effect	Significance test from the essential oil of Rosemary				
	SC	Degrees of freedom	MC	F	P
Ord. origine	7594434	1	7594434	44.47030	0.000002
Application	1907637	4	476909	2.79261	0.054210
Error	3415508	20	170775		

**Discussion:**

The diagnosis of varroa by the biological method allowed us to estimate the approximate number of parasites in each colony and then calculate the degree of initial infestation, it varies between 3.96% and 42.76%. These results are compared to those established by Robaux (1986), we recorded well.

- 02 colonies (H4 and H8) with an infection rate of less than 5%, for which no danger and no emergency treatment is needed.
- 01 colony (H6) with an infection rate of between 5 and 10%, the colony is seriously undermined.
- 05 colonies (H1, H2, H3, H5, and H9) have an infestation rates between 10 and 20%, the colony is highly reached.

- 03 colonies (H7, H10 and H13) have an infestation rates between 20 and 30% probable collapse of the colony.

- 04 colonies (H11, H12, H14, and H15) with an infestation rate of more than 30%, the colony is considered lost. Faced with this situation, treatment is required immediately.

Regarding the average rate of each batch, lot 1 and lot 2 respectively show an infection rate of 11.99 % and 14.67 %, the degree of infestation is high thus resulting in a heavy infestation. In this situation, recourse to treatment is necessary to prevent the collapse or loss of the colonies. For witness batch, the average infection rate is 33.24 %.

It is noteworthy that after the treatment, the infection rate increased from 11.99% to 0.31% or a decrease of 11.68% for the rosemary and 14.67% to 0.45% is a decrease of 14.22% mint. For lot 3, we recorded significant varroa mortality. The final infestation rate is of the order of 22.1%.

During the two treatments for Lot 1 and Lot 2, it is clear that the essential oil of rosemary and mint have generated significant mortality in the first application (31 May to 04 June). This period coincided with the emergence or hatching young bees in their cells and exposure to essential oils. Observations of mortality rates in the essential oil therapy rosemary showed a significant drop dead varroa during this period, it is 62.9%. By against the essential oil of mint caused low mortality, which is 54.96%. The control treatment by Apiguard led only small mite mortality. It is 2.49% (Lot 1) and 1.46% (lot 2). The high summer temperatures have made the mites become more vulnerable. This confirms the results of Robaux (1986). This author found that during this period the mortality rate can reach 68%. Statistical analyzes showed that the variance is so significant between the rosemary and the treatment period but not significant for mint. The efficiency rate is optimal for both lots; the effectiveness is assessed at 95.89% on average for the rosemary and 97.38% on average for the mint. It thus confirms that established by Borneck and Robin (1989) of between 99.38% and 99.72% over a period of 42 days. The low efficiency of the treatment is mainly related to the presence of capped brood. It prevents the introduction of the active material in the cells [1-4]. Various studies of over 150 essential oils to test their effectiveness, have mounted that only thyme oil, sage and oregano showed good efficiency in their applications [10]. Moreover, According Maggi *et al* 2010, *Syzygium aromaticum* clove oil also showed a proven toxicity *varroa destructor* but toxicity *Apis mellifera* has not been evaluated. Also Kotwal *et al* (2012 ) showed that the essential oils vary in their effectiveness depending on the molecules and their dosages used . However, their use in combination with several essential oils and other active ingredients could provide solutions in the management of the fight against *Varroa destructor* and resistant strains.

in 2013, Ghalem *et al* used four essential oils of rosemary (*Rosmarinus officinalis*), mint (*Mentha viridis*), laurel (*Laurusnobilis*) and thyme (*Tymus pubescens*) against *Varroa destructor* in the North African bee *Apis mellifera intermissa*. After treatment, they found that the initial rate of around 7%, reflecting the severity of the parasitic disease has decreased significantly at rates between 0.70% and 5.15%. Statistical analysis revealed significant differences between the three types of treatments (rosemary, mint and bay leaves). This difference shows that there was a treatment effect on this parasitic activity. Treatment with laurel oil has been more successful in reducing the development of varroa. Comparing the varroa mortality highlights the importance of effective treatment.

In 2013, Berkani- Ghalem and *al*, also worked on the population dynamics of *Varroa destructor* ectoparasite in northern Algeria; where there's the northern climatic differences south. They found that more bee colonies are placed in high temperature areas and varroa mites do not survive. Finally, prospecting numerous plants under acaricide should be performed in all areas and beekeeping, it would be desirable to do other tests based on natural products to fight against varroa which to draw satisfactory results.

#### Conclusion:

Since the emergence of new diseases such infestation of varroa, the practice of beekeeping has become more complicated and demanding. For this fight against diseases is of paramount importance in our world. The presence of *varroa destructor* was detected by a simple natural diagnosis which also assessed the degree of parasitosis in bee colonies. Such a diagnosis is not appropriate in the presence of brood [15]; and revealed in our study 11.99% infestation rate and 14.67% respectively for lot1 and lot 2. This rate is between 10% and 20% which means that colonies strongly affected and require treatment. Rosemary and mint, showed relatively good efficiency which could be due to the concentration of oils. However, these have a short duration of action in the colony and therefore any mites that pass this treatment period without damage reproduce and make it necessary to repeat the cure shortly after. No treatment has been able to achieve a total efficiency, but it is clear that the two essential oil appear to have greatly reduced the rate of final infestation to 0.31% for the rosemary and 0.45% for the menthe. They were selected as acaricides effective in short-term fight against varroa, tolerated by bees and preserve the quality of bee products. The high temperature climatic conditions and the brood reduction constituted favorable factors for the eradication of the parasite; thus increasing the chances of therapeutic success.

Finally, it is important that beekeepers know the level of infestation by varroa mites in honey bee colony before it becomes too high. Therefore, they must constantly monitor levels of infestation in their colonies and regularly apply treatments to limit the damage caused by infestations. The implementation of these essential oils, considered organic and natural, has not hampered the healthy development of the colonies. For this it is recommended treatments, based on essential oils from medicinal plants. They allowed a decrease in virulence of the parasite in the apiary. That their success will be maximized, certain parameters must be studied extensively especially in the case of Algeria namely: Analysis of essential oil residues in honey and wax and in the study of habituation that can occur in the short or long term after treatment administration [5].

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